Short Communication

mTOR Inhibition Alleviates L-DOPA-Induced Dyskinesia in Parkinsonian Rats

Mickael Decressac* and Anders Björklund
Wallenberg Neuroscience Center, Department of Experimental Medical Sciences, Lund University, Lund, Sweden

Abstract. The development of dyskinesia upon chronic L-DOPA treatment is a major complication for the management of the motor symptoms in Parkinson’s disease (PD) patients. Efforts are made to understand the underlying mechanisms and identify targets for the pharmacological alleviation of dyskinesia without affecting the therapeutic effect of L-DOPA. Previous studies have shown that the mTOR pathway is hyperactive in dyskinesia as a consequence of D1 receptor hypersensitivity. We investigated the effect of the FDA-approved mTOR inhibitor Temsirolimus (CCI-779), currently used in the clinic, on the development of LID and on the severity of already established LID in hemi-parkinsonian rats. Systemic delivery of CCI-779 prevented the development of LID and significantly alleviated the severity of dyskinesia in L-DOPA-primed animals. This was associated with a reduced activation of the mTOR pathway in striatal medium spiny neurons. Drugs with mTOR inhibiting activity that are actively developed in cancer research may be of interest for the management of LID in PD patients.

Keywords: mTOR inhibition, dyskinesia, Parkinson’s disease

L-DOPA therapy remains the most efficacious medication for the management of motor symptoms in the early stage of Parkinson’s disease (PD). However, it does not counteract the pathogenic process, and as the disease progresses dopamine fluctuation triggers troublesome side effects mainly characterized by the appearance of abnormal involuntary movement, i.e. dyskinesias [1]. The occurrence of these severe motor complications seriously hampers the use of L-DOPA in the clinic, justifying the need for a better understanding of the physiopathological mechanisms underlying this maladaptive change.

Notably, enhanced extracellular signal-regulated kinases (ERK) and mammalian target of rapamycin (mTOR) signalling has been strongly associated with the emergence of LID, resulting from the development of hypersensitivity in the D1 receptor expressing striatal neurons [2–4]. In this line, a recent study reported that transgenic mice lacking Rhes, a striatal-enriched small G protein activating mTOR, showed reduced dyskinesia [5]. In addition, Santini and colleagues showed that a similar anti-dyskinetic effect could be achieved by treatment with the mTORC1 inhibitor rapamycin in the 6-OHDA mouse model of PD [3]. New derivatives of rapamycin (“rapalog”) with better pharmacological profiles and reduced side effects in humans are currently in use for the treatment of cancer [6], and therefore deserves to be tested in this context [7]. In the present study, we examined whether the rapamycin ester CCI-779 (Temsirolimus), which is currently in use in the clinic, can afford an anti-dyskinetic effect in the standard 6-OHDA rat model of PD.

Adult female Sprague Dawley rats, 225–250 g at the time of surgery, were housed two to three per cage with ad libitum access to food and water during a 12 hours light/dark cycle. All procedures were approved...
and conducted in accordance with guidelines set by the Ethical Committee for the use of laboratory animals in the Lund-Malmö region and the European Ethical Committee (86/609 EEC). All surgical procedures were performed under general anesthesia using a 20:1 mixture of fentanyl citrate (Fentanyl) mefedrin hydrochloride (Dormitor) (Apoteksbolaget, Sweden) injected i.p. Rats were placed in a stereotaxic frame (Stoelting) and 3 μl of 6-OHDA (3.5 μg/μl free base dissolved in a solution of 0.2 mg/ml L-ascorbic acid in 0.9% w/v NaCl) (Sigma) was injected using a 10 μl Hamilton syringe fitted with a glass capillary (outer diameter of 250 μm) (0.2 μl/min), in the medial forebrain bundle (MFB) at the following coordinates (flat skull): anterio-posterior: −4.4 mm, medio-lateral: −1.1 mm, dorso-ventral: −7.8 mm.

Three weeks after 6-OHDA injection into the MFB, the rats were screened behaviorally in the amphetamine-induced rotation test (2.5 mg/kg, i.p.) and the cylinder test, as described previously [8]. Animals exhibiting ≥7 ipsilateral full body turns/min and less than 20% of forelimb use were selected for study of L-DOPA-induced dyskinesia (LID). We explored the effect of CCI-779 on 1) the development of LID, and 2) on the severity of established LID, using the respective experimental designs:

1. Animals (n = 8 per group) were treated daily for 3 weeks with L-DOPA methyl ester (6 mg/kg) together with a peripheral DOPA-decarboxylase inhibitor, benserazide (10 mg/kg). CCI-779 was administered at a dose of 20 mg/kg, i.p., 3 times per week 1 hour before L-DOPA [9]. Injections were performed until a stable level of abnormal involuntary movements (AIMs) was achieved in the control vehicle-treated group (5% ethanol, 0.15 M NaCl, 5% Tween 20, 5% PEG 400) (3 weeks) (see Fig. 1 for experimental design).

2. Animals (n = 8 per group) were treated daily for 3 weeks with L-DOPA (6 mg/kg) and benserazide (10 mg/kg), until a stable level of AIMs was achieved, then allocated into two well-balanced groups and received either L-DOPA + CCI-779 or the vehicle 3 times during week 4. AIMs were scored after L-DOPA+treatment (CCI-779 or vehicle) on week 5 (see Fig. 1 for experimental design).

In both experimental designs, cylinder test was performed at various times to assess the effect of the treatment on the anti-akinetic effect of L-DOPA (see Fig. 1 for experimental design).

The rats were scored every 20 min for 140 min after L-DOPA injection (or as long as AIMs were observed) by an observer blinded to the identity of the animals and using the rat dyskinesia scale of Cenci et al., as described in detail previously [10, 11]. On the day of sacrifice, rats received their assigned treatment and were killed 1 hour after L-DOPA injection (6 mg/kg) in order to assess the effect of CCI-779 on phospho-S6 expression in the striatum. S6 protein is a component of the ribosome that is phosphorylated downstream of the
PI3K/mTOR, MAPK and PKA pathways, and therefore in the striatal D1-expressing neurons in dyskinetic animals following L-DOPA injection [3, 7, 12].

Preparation of the sections and histological procedures was performed as described [8]. Immunohistochemical stainings were performed on free-floating sections using antibodies raised against tyrosine hydroxylase (TH) (rabbit, 1:1500; Chemicon) or phospho-S6 (Ser235/236) (rabbit, 1:300; Cell Signaling) and staining was visualized using 3, 3-diaminobenzidine (DAB) as a chromogen. High-resolution images were captured from two sections, at +0.20 and −0.30 mm from bregma, using a Scanscope GL system with Imagescope v8.2 software. A 0.8 × 0.8 mm² area from the medial, lateral and central part of the striatum was selected for analysis using the ImageJ software. The intense staining and low background allowed for software-automated calculation of the total number of phospho-S6-positive cells based on optical density upon defining the threshold for specific signal [8].

All statistics were conducted using the GraphPad Prism software (version 5.0). All values are presented as mean ± standard error of mean (SEM). Comparison between experimental groups was performed using a Student t-test (Fig. 2F). Cylinder test and dyskinesia scores were analyzed using a one-way ANOVA followed by Tukey’s post hoc test or a two-way ANOVA followed by Bonferroni post hoc test (with time and treatment as variables). Statistical significance was set at P < 0.05.

Consistent with the behavioural impairment observed in the cylinder (pre-treatment in Fig. 2D) and amphetamine-induced rotation test (see Material and methods), all animals in both experimental designs showed profound loss of nigral DA neurons and striatal innervation (>90% compared to contralateral side). Drug treatment had no effect on either cell survival or striatal innervation density (data not shown).

Treatment with CCI-779 during the 3-week priming period blocked the development of LID. Thus, the animals that had received injections of CCI-779 (20 mg/kg, 3 times/week) developed only mild dyskinesia (13 ± 2, after 3 weeks) compared to the severe dyskinesia seen in the vehicle-treated animals (48 ± 4, after 3 weeks) (time × treatment: F4,184 = 17.07, P < 0.001; treatment: F1,44 = 73.50, P < 0.001; time: F4,184 = 68.63, P < 0.001; n = 8 per group) (Fig. 2A). Moreover, when similar CCI-779 treatment was given to primed animals (3 injections of 20 mg/kg during the 4th week of L-DOPA treatment), we observed a robust anti-dyskinetic effect of CCI-779 (time × treatment: F9,126 = 27.94, P < 0.001; treatment: F1,44 = 69.23, P < 0.001; time: F9,126 = 99.31, P < 0.001; n = 8 per group) (Fig. 2B) as shown by the significant reduction (−64%) in AIMS score (16 ± 3 compared to the level of dyskinesias seen at the start of CCI-779 treatment (44 ± 4) and to the vehicle treated group (43 ± 5)) (Fig. 2C). In addition, CCI-779 treatment did not interfere with the anti-akinetic effect of L-DOPA as vehicle and drug-treated groups performed equally in the cylinder test in both experimental designs (treatment: F1,44 = 9.72, P < 0.001; n = 8 per group) (Fig. 2D).

Consistent with these findings, histological analysis showed a marked reduction in the number of phospho-S6-positive striatal neurons in rats treated with CCI-779 (74 ± 16 cells in experiment 1; 119 ± 20 cells in experiments 2) compared to the control group (282 ± 38 cells in experiment 1; 311 ± 29 cells in experiment 2) (Fig. 2E, F) (P < 0.001), representing a 74% reduction in experiment 1 and a 62% reduction in experiment 2.

Genetic or pharmacological inhibition of the mTOR complex 1 has recently been shown to afford anti-dyskinetic effect. In the present study, we confirm the observation of Santini et al. that mTOR inhibition is effective in preventing the development of L-DOPA induced dyskinesia [3]. In addition, we show that the severity of already established dyskinesia in chronically L-DOPA treated animals can be blocked by inhibition of mTOR. Notably, this effect was obtained 1) in the most commonly used model of parkinsonism-related dyskinesia, i.e. the 6-OHDA MFB lesion in rats, and 2) using a rapamycin ester that is currently used in the clinic for treatment of cancer. Consistent with these findings, we observed a marked decrease in the number of striatal cells expressing intense phospho-S6 immunoreactivity in response to L-DOPA, supporting the correlation between low mTOR activation and magnitude of LID [3].

These results point to mTOR as an interesting therapeutic target for anti-dyskinetic therapy. This is further supported by the observation that mTOR inhibition induces the anti-dyskinetic effect without any interference with the therapeutic, anti-akinetic action of L-DOPA treatment [3, 5] (using the cylinder test in the present study), suggesting that the therapeutic benefits of L-DOPA on motor function are not mediated by the mTOR pathway. Despite being efficacious in the present acute design and tolerated for long-term use in cancer treatment, chronic inhibition of mTOR in the context of PD-related dyskinesia may not represent a...
Fig. 2. CCI-779 prevents the development and reduces established L-DOPA induced dyskinesia in 6-OHDA-lesioned rats. A: Time course of changes in dyskinesia induced by daily L-DOPA treatment (6 mg/kg plus benserazide 12 mg/kg, s.c.) in combination with either CCI-779 (20 mg/kg, given 3 times/week; black circles) or vehicle (open circles) over a 3-week priming period. Two-way ANOVA test followed by Bonferroni post hoc test. *, P < 0.001; compared to vehicle-treated group (n = 8 per group). B: Effect of CCI-779 in L-DOPA-primed animals (6 mg/kg plus benserazide, daily for 3 weeks) followed by treatment with CCI-779 during the 4th week of L-DOPA treatment. Time course of L-DOPA induced dyskinesia in animals given CCI-779 (20 mg/kg; black circles) or vehicle (open circles) over the 3-hour test period, performed in week 5. Two-way ANOVA followed by Bonferroni post hoc test, *, P < 0.001; compared to the vehicle-treated group (n = 8 per group). C: Total AIMS score in L-DOPA-primed, dyskinetic rats at the start of CCI-779 treatment, i.e. at the end of week 3, and after treatment with CCI-779 (black bar) or vehicle treatment (open bar) performed in week 5. One-way ANOVA followed by Tukey’s post hoc test; #: P < 0.01; compared to the pre-treatment group. *, P < 0.001 compared to the vehicle-treated group (n = 8 per group). D: Cylinder test was performed 3 weeks after 6-OHDA lesion (before L-DOPA priming period) and at the end of the treatment period (see Fig. 1). CCI-779 did not interfere with the anti-akinetic effect of L-DOPA. Two-way ANOVA followed by Bonferroni post hoc test, *, P < 0.001; compared to pre-treatment (n = 8 per group). E-F: Immunostaining of phospho-S6 in the striatum of animals from experiment 1 and 2, treated with CCI-779 or vehicle, given together with L-DOPA 1 hour before sacrifice (E). Scale bar: 200 μm. Quantification of the number of phospho-S6-positive striatal cells (F); *, P < 0.001; Student’s t-test (n = 8 per group).

In conclusion, and in line with previous reports, our data provide a proof-of-concept that inhibition of the mTOR pathway in D1-expressing striatal neurons represents a promising approach to alleviate LID. Interestingly, Subramanian and colleagues recently identified Rhes, a striatal-enriched small G-protein, as a potentially interesting target to alleviate dyskinesia. Experiments performed in Rhes lacking mice showed that inhibition of this protein reduced LID without altering the symptomatic benefit of L-DOPA [5] pointing to Rhes as a candidate for the development of specific inhibitors as tools for valuable therapeutic interventions in the context of LID.
ACKNOWLEDGMENTS

The study was supported by grants from the Swedish Research Council (grant no. 04X-3874 and the BAGADILICO program), and Swedish Foundation for Parkinson’s disease. The authors thank Ulla Jarl, Bengt Mattsson and Michael Sparrenius for excellent technical assistance. CCI-779 (Temsirolimus) was provided by Pfizer.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES